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Vaccination With Autoreactive T Cell Clones in Multiple Sclerosis: Overview of Immunological and Clinical Data

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Although the etiology and pathogenesis of Multiple Sclerosis (MS) remain elusive, accumulating evidence indicates that MS is a chronic inflammatory disease with an autoimmune component, mediated by autoreactive T lymphocytes specific for myelin antigens. The triggering T cell autoantigen has not been identified yet, but recent immunological studies in MS and parallel experiments in experimental allergic encephalomyelitis (EAE), the animal model of MS, have indicated that myelin basic protein (MBP) can be considered as one of the major candidate autoantigens in the pathogenesis of the disease. Based on these observations, several therapeutic strategies have been developed aimed at the specific elimination or inactivation of MBP reactive T cells in MS. One of these approaches involves the immunization of MS patients with autologous attenuated autoreactive T cells to induce an immune response specifically targeted at these autoreactive T cells. This method, termed T cell vaccination, has been shown to prevent and treat EAE. We have recently conducted a pilot trial of T cell vaccination in a limited group of MS patients to evaluate the immunological responses to the injected cells. The data obtained indicate that this type of vaccination induces an effective anti-clonotypic T cell response leading to a specific depletion of circulating MBP reactive T cells. Preliminary data on the clinical effects are promising, encouraging further clinical trials. © 1996 Wiley-Liss, Inc.

Key words: autoimmunity, autoreactive lymphocytes, immunotherapy, myelin basic protein

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) leading to demyelination (ffrench-Constant, 1994). In the CNS, the demyelination is accompanied by an inflammatory response, most likely initiated by perivascular infiltrates of $CD4^+$ T lymphocytes and activated macrophages (Prineas and Wright, 1978; Raine 1994). The levels of several inflammatory cytokines are increased in the plaques, and the expression of TNF-α and IL-4 is increased in MS lesions (Canella and Raine, 1995; Selmaj et al., 1991; Hofman et al., 1989). Together with the observations of an increased expression of MHC class II molecules on reactive astrocytes, the infiltration of γδ T cells and activated αβ T cells in MS lesions and the increased expression of IL-2 receptors, these findings are reminiscent of an ongoing immune reaction in the brain compartment of MS patients (Stinissen et al., 1996). Since similar immune abnormalities have been observed in other autoimmune conditions such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), it was argued that MS is an autoimmune disease mediated by T helper cells directed at CNS myelin components (Hafler and Weiner, 1989; Wucherpfennig et al., 1991). Furthermore, as seen in other autoimmune conditions, an increased susceptibility to MS is associated with particular MHC class II alleles. For example, Caucasian MS patients overexpress the HLA-DR2 allele (reviewed by Olerup et al., 1991). The likelihood of an autoimmune disease process in MS is further suggested by the similarities with experimental allergic encephalomyelitis (EAE), an animal model for MS, sharing many clinical and histological features with MS (reviewed by Zamvil and Steinman, 1990). EAE is an experimental autoimmune disease characterized by focal areas of inflammation and demyelination in the CNS, induced by injecting myelin or myelin components in susceptible animals. The T cell mediated autoimmune nature of EAE has been shown experimentally by the observation that the disease can be adoptively transferred to naive animals by myelin

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reactive T cells (Ben-Nun et al., 1981a,b; Vandenbark et al., 1985).

There is still no effective cure for MS. Patients undergoing relapses are treated with nonspecific immune suppressive drugs (Ebers, 1994). Based on recent insights in the disease mechanisms, new therapeutic strategies have being proposed, and some of them have indicated to have potential therapeutic effects in a clinical setting. Interferon-β (IF-β) and copolymer-1 (COP-1) were recently tested in multicenter phase III studies and were found to be effective in reducing the relapse rate in relapsing-remitting patients (IFNB MS Study Group, 1993; Johnson et al., 1995). Furthermore, IF-β was also shown to have beneficial effects on the brain lesions themselves as illustrated by magnetic resonance imaging (MRI) (Paty et al., 1993). Other immunotherapeutic approaches are more specifically targeted at the pathogenic autoimmune T cells. One of these strategies is T cell vaccination, a procedure which is thought to upregulate the regulatory networks that control autoreactive T cells. In a recent pilot study, we have injected autologous attenuated myelin basic protein (MBP) reactive T cell clones in eight MS patients.

MYELIN-REACTIVE T CELLS IN MULTIPLE SCLEROSIS

Since myelin is considered to be one of the primary targets of the autoreactive T cells in MS, several myelin components have been considered as candidate autoantigens in MS. This list includes MBP, proteolipid protein (PLP), myelin associated glycoprotein (MAG), and myelin oligodendrocyte glycoprotein (MOG). The evidence illustrating the role of some of these candidate autoantigens mainly comes from studies in the EAE model. The encephalitogenic potential of MBP and proteolipid protein (PLP) antigens has been clearly demonstrated in EAE and the encephalitogenic epitopes were defined in various animal strains (Tuohy et al., 1988). For instance, the MBP 89-100, Ac 1-10, and 69-86 epitopes are encephalitogenic in SJL/J mice, PL/J, or B10.PL mice, and Lewis rats, respectively (reviewed by Martin and McFarland, 1995). In general, the immunodominant regions coincide with the encephalitogenic epitopes. PLP and MBP specific Th1 cells isolated from EAE animals are able to transfer the disease to naive animals (Vandenbark et al., 1985; Zamvil et al., 1986; Kuchroo et al., 1992). Moreover, the encephalitogenic T cells isolated from various animal strains express a highly restricted T cell receptor (TCR) V gene repertoire (Acha-Orbea et al., 1989). For example, Ac1-10 MBP specific T cells from PL/J mice express Vβ8.2-Vα2.3 or Vβ8.2-Vα4.2 TCR's. Furthermore, encephalitogenic T cells isolated from Lewis rats use similar Vα/Vβ pairs, even though they recognize a different MBP epitope. The restricted TCR repertoire of the encephalitogenic T cells in EAE offers possibilities for TCR directed therapies in these animals (Acha-Orbea et al., 1988).

MBP and PLP antigens have been evaluated as candidate autoantigens in MS. MBP specific T cells can be isolated from most individuals (both MS patients and control subjects) by repeated in vitro stimulation with MBP, indicating that MBP specific T cells are part of the normal T cell repertoire. However it is not possible in humans to correlate the in vitro reactivity to myelin antigens with the pathogenic effects in vivo. Nevertheless, several important clues can be obtained from the analysis of the precursor frequency, activation status, epitope reactivity, HLA restriction, and TCR expression of these cells. For instance, since immunodominant epitopes are generally also encephalitogenic in EAE, the identification of these epitopes in MS is of considerable interest. Two immunodominant epitopes were identified in the MBP molecule, both for MS patients and control subjects: 84-102 and 143-168 (Ota et al., 1990; Pete et al., 1990; Liblau et al., 1991; Martin et al., 1990, 1992; Zhang et al., 1992;). Remarkably, the middle region of the MBP molecule is encephalitogenic in Lewis rats and SJL/J mice as well. Furthermore, the frequency of the T cells recognizing this 84-102 epitope was higher in $DR2^+$ MS patients than in control subjects (Zhang et al., 1992). HLA-DR molecules, and to a lesser extent DQ antigens, can present the immunodominant 84-102 peptide to T cells (Chou et al., 1989; Jaraquemada et al., 1990; Martin et al., 1990), and interestingly, this immunodominant peptide 84-102 binds with the highest affinity to the disease associated DRB1*1501 (DR2,Dw2) molecules (Wucherpfennig et al., 1994a,b).

Using limiting dilution analysis no major differences were observed in the precursor frequency of MBP reactive T cells between MS patients and control subjects (Zhang et al., 1992). The T cell frequency varies substantially among both MS patients and control individuals, with an average of one MBP reactive T cell per million mononuclear cells in peripheral blood. However, when IL-2 is used as a primary stimulus for the blood mononuclear cells instead of MBP, the precursor frequency of MBP reactive T cells is higher in most MS patients as compared to control subjects (Zhang et al., 1994). The higher frequency of IL-2 responsive, or IL-2 receptor positive, MBP reactive T cells probably reflects an increased number of activated MBP specific T cells in MS patients, since activated T cells express the IL-2 receptor at higher concentrations as compared to resting T cells. In addition, the precursor frequency of IL-2 responsive (activated) MBP specific T cells in cerebrospinal fluid of MS patients is increased as compared to control subjects, thus indicating increased levels of acti-

vated MBP-specific T cells in the CNS of MS patients (Zhang et al., 1994). The observed expansion of in vivo activated MBP reactive T cells in the CNS further supports the role of these cells in the disease process. The IL-2 responsive activated T cells recognize the same immunodominant regions of MBP as the over all MBP specific T cells present in circulation. A similar expansion was observed for activated PLP specific T cells in the CSF (Zhang et al., 1994). Accumulation of activated MBP reactive T cells in CSF of MS patients was also observed by other investigators (Allegreta et al., 1990; Chou et al., 1992).

In conclusion, no substantial differences in frequency, epitope reactivity and HLA-restriction of MBP specific T cells were found between MS patients and healthy subjects. In MS patients, however, activated MBP specific T cells accumulate in the CNS, indicating the potential role of these autoimmune cells in the pathogenesis of the disease. The immunodominant epitope 84-102 is efficiently presented by the disease associated HLA-DR2 molecules. This peptide might be important in the disease process because it corresponds to an encephalitogenic region in several rodent strains.

In the EAE model, encephalitogenic T cells express a restricted TCR repertoire. In MS, some authors have described a preferential use of Vβ5, for MBP specific clones derived from different MS patients (Kotzin et al., 1991; Oksenberg et al., 1993), while no or less inter-individual V gene sharing was found in other studies (Ben-Nun et al., 1991; Wucherpfennig et al., 1994; Vandevyver et al., 1995). Recent studies from our and other groups and a collaborative analysis in which large panels of MBP reactive T cell clones were evaluated demonstrated a heterogeneous TCR V gene usage of MBP specific T cell clones among patients with MS and control individuals (Wucherpfennig et al., 1994; Vandevyver et al., 1995; Hafler et al., 1996). However, within a given patient a restricted number of TCR V gene elements can be observed. This restricted repertoire is compatible with a clonal expansion in these patients as demonstrated by identical CDR3 sequences of the T cell receptors of these clones. In some patients one or two clones dominate the T cell responses towards MBP. Some of the clonally expanded populations persist for several years (Meinl et al., 1993; Salvetti et al., 1993, Hohlfeld et al., 1995). Although limited clonal expansion was also observed in some control subjects the TCR repertoire in general was more heterogeneous as compared to patients with MS (Vandevyver et al., 1995; Hafler et al., 1996).

The data accumulated so far support that clonally expanded MBP specific T cell populations persist in the periphery and brain of MS patients, indicating that these cells are stimulated in vivo, and providing further evidence for their potential pathogenic involvement in the disease. Current data also indicate that the TCR repertoire of the MBP specific T cells may be biased in a given patient, but that the repertoire varies among patients with MS. More research is necessary to resolve this issue of inter-individual V gene restriction. This question however is of utmost importance, since specific therapies might be designed to target specific TCR Vα or Vβ segments. If the repertoire of the MBP specific T cells is more diverse, such therapies might still be effective if tailored for each individual patient.

MBP specific T cells are suspected to become activated in the periphery of MS patients. Once activated, these T cells rapidly pass the blood-brain barrier where they may initiate the autoimmune cascade (Hafler et al., 1987), involving recruitment of B cells, macrophages and γδ T cells, secretion of pro-inflammatory cytokines such as TNF and IFN-γ and activation of glia cells. Myelin reactive T cells can be activated by myelin breakdown products presented by brain specific antigen presenting cells. Myelin breakdown is probably the result of the combined action of cytotoxic cells including macrophages and γδ T cells, demyelinating antibodies, cytokines (e.g., TNF), and cytotoxic mediators (Stinissen et al., 1996). It remains an open question how MBP autoreactive T cells are activated in the periphery, as there is no contact with the CNS myelin. One of the potential mechanisms may involve molecular mimicry with viral antigens or activation by bacterial or viral superantigens (Zhang et al., 1995a). These pathways may activate autoreactive T cells in some individuals and lead to autoimmunity in a minority of the sensitized subjects. Several regulatory mechanisms that control activated autoreactive T cells have been postulated. These mechanisms may involve antigen specific suppresser cells and anti-idiotypic T cell responses (Antel et al., 1979; Cohen, 1992). Indeed, there is some experimental evidence indicating defective suppressive mechanisms in MS patients. In this situation, autoreactive T cells are no longer suppressed or properly controlled when activated, and may induce autoimmune pathogenesis. Therefore, autoimmune disease could be the result of a defective regulation of natural autoimmunity (Cohen, 1992).

T CELL DIRECTED THERAPIES AND THE CONCEPT OF T CELL VACCINATION

Immune intervention targeted at the pathogenic autoimmune T cells may lead to specific therapies for MS and other T cell mediated autoimmune diseases (Steinman, 1991). In some approaches, T cell tolerance induction towards MBP is a primary goal. T cell tolerization has been induced by using altered MBP 84-102 peptide ligands that induce T cell anergy, or induce a Th1 to Th2

shift (Adorini et al., 1992). Oral tolerance induction by feeding MBP or myelin is an alternative procedure to induce organ specific tolerance by active suppression or clonal anergy (Weiner et al., 1993). In other strategies, the TCR of autoreactive T cells is the main target. This may be a perfect attacking point, as this marker is able to distinguish the pathogenic T cells from other unrelated T cells. To be successful, however, the pathogenic T cells must express an homogeneous TCR repertoire. This is the case in EAE, where encephalitogenic MBP-specific T cells use restricted TCR Vβ gene segments. Various therapeutic strategies have been designed to target the TCR by monoclonal antibodies to the Vβ gene products which are preferentially used by encephalitogenic T cells or by vaccination with a peptide matching the CDR2 region of the responsible Vβ gene (Vandenbark et al., 1989, 1993; Howell et al., 1989). These studies were remarkably successful in preventing the development of EAE in susceptible animals and some of these strategies have been tested in human autoimmune diseases as well (Chou et al., 1994). The trials are based on the observation that a limited TCR Vβ repertoire is preferentially used by MBP specific T cells in MS. However, as discussed before, most studies do not support the preferential TCR usage of MBP specific T cells in MS. MBP autoreactive T cell clones show a heterogeneous TCR Vβ gene which is relatively restricted in individual MS patients. A treatment designed to target certain TCR V gene product(s) may therefore be useful in a single patient only, which hampers its clinical applicability.

Immunization with complete pathogenic T cells is another method which proved to be effective in the EAE model. When researchers in the laboratory of Irun Cohen immunized rats with attenuated encephalitogenic T cells, the vaccinated animals became resistant to subsequent induction of EAE by MBP (Ben-Nun et al., 1981). This procedure was termed T cell vaccination in analogy with traditional microbial vaccination with attenuated infectious agents. The protection was found to be specific and long lasting. Interestingly, only activated T cells were effective in vaccination. The resistance to the disease could be transferred to other animals by T cells from vaccinated animals that were raised against the immunizing clones (Holoshitz et al., 1985). Experiments in EAE further demonstrated that T cell vaccination induces or augments the regulatory networks to specifically suppress the eliciting autoreactive T cells (Lider et al., 1988). T cell vaccination has been found to be applicable in many animal models of autoimmune diseases including adjuvant arthritis and non-obese diabetic mice. As discussed below, the mechanism of T cell vaccination involves the anti-idiotypic T cell regulatory network that controls autoreactive T cells.

T CELL VACCINATION IN MS

Design of the Study and Vaccine Development

Based on the potential pathological role of MBP specific T cells in MS and encouraged by the successful treatment of EAE by T cell vaccination, a pilot trial of T cell vaccination was conducted in MS patients (Zhang et al., 1993; Medaer et al., 1995). Eight MS patients were immunized with irradiated autologous MBP-specific T cell clones. The vaccine cells were isolated and prepared for vaccination as illustrated in Figure 1. The T cell clones were isolated in a two-step procedure. In the first step, MBP reactive T cell lines were obtained by stimulation of peripheral blood mononuclear cells (PBMC) with MBP in a microtiter plate format. The MBP reactive lines were identified by classical cell proliferation assays. Using Poison statistics the precursor frequency of MBP reactive T cells was determined. In the second step, these lines are further cloned by plating out at low cell densities (0.3 or 1 cell per well) and stimulating the cells with PHA and irradiated PBMC as feeder cells. In this way, highly purified T cell clones are obtained, which can be further expanded by repeated stimulation with MBP pulsed PBMC as antigen presenting cells and addition of recombinant IL-2. This T cell cloning method is very critical in the vaccine preparation since only highly purified T cell clones can be expanded to the number of cells needed for vaccination. The clones were also characterized for their epitope reactivity and TCR characteristics (V gene usage and CDR3 DNA sequences). The T cell clones used for vaccination were selected on the basis of their reactivity to the two immunodominant regions of human MBP, which dominated the T cell responses to MBP in these individuals. The vaccine clones were activated with MBP pulsed autologous PBMC cells 1 week prior to vaccination, and irradiated before vaccination (6,000 Rad, Cs-source). The cells were injected subcutaneously in both arms at a dose of 10–15 million cells/clone. All patients were vaccinated three times with an interval of 2–4 months. After each vaccination, the patients were monitored for routine blood parameters, immunological responses towards the vaccine, frequency of the MBP reactive T cells, and for their clinical characteristics.

Toxicity and Immunological Responses

Our study demonstrated that subcutaneous inoculations of the autologous vaccine clones are well tolerated and cause no adverse effects (Zhang et al., 1993). Administration of the vaccines induced substantial anti-vaccine or anti-clonotypic T cell responses specifically to the vaccine clones, which were accompanied by a specific depletion of circulating MBP reactive T cells in all recipients (Zhang et al., 1993). This depletion of MBP

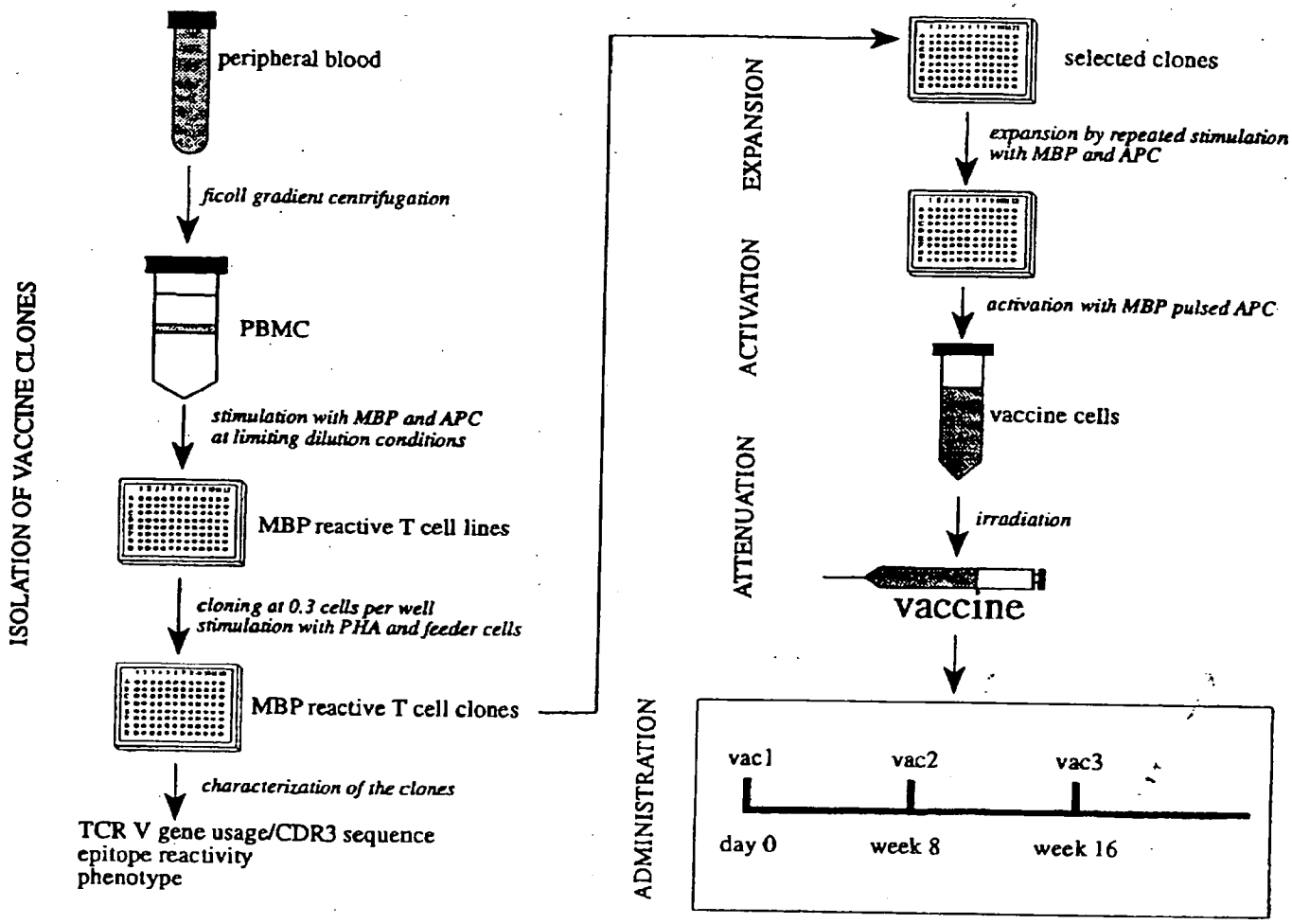

Fig. 1. Overview of vaccine preparation. Peripheral blood mononuclear cells (PBMC) are purified from heparinized peripheral blood by Ficoll gradient centrifugation. The PBMC are plated at different cell densities and stimulated with MBP in 96-well microtiter plates. After 1 week, the cultures are restimulated with MBP pulsed antigen presenting cells (APC). At day 14 the cultures are screened using a classical proliferation assay, and MBP reactive T cell lines are identified. The limiting dilution conditions allow determination of the precursor frequency of the MBP specific T cells. MBP reactive T cell clones are then obtained by plating out the cell lines at 0.3 or 1 cell per well and stimulation with PHA and irradiated feeder cells. These clones are characterized for their epitope reactivity and TCR gene rearrangements. The selected clones are then expanded to high cell numbers (typically 40–60 million cells) by repeated stimulation with MBP pulsed antigen presenting cells. To prepare the cells for vaccination, the clones are activated by MBP 8 days prior to vaccination, and then attenuated by irradiation (6,000 Rads, Cs-source). The vaccine clones are injected at a dose of 10–15 million cells per clone. Three inoculations are performed with an interval of 2 months.

reactive T cells appears to be the direct effect of anti-clonotypic T cells, since $CD8^+$ anti-clonotypic T cell lines isolated from vaccinated patients specifically lysed the autologous vaccine clones in a MHC class I restricted fashion. The anti-clonotypic T cells were generated from the vaccinated patients by repeated stimulation with irradiated vaccine clones and selected on the basis of their specific suppressive effects on the antigen induced proliferation of the vaccine clones. In addition to their cytotoxic activity towards the vaccine cells, the anti-clonotypic T cells also proliferated in response to irradiated vaccine cells. Although the vast majority of isolated anti-clonotypic T cells expressed the CD8 phenotype, a few clones were $CD4^+$. These $CD4^+$ anti-clonotypic clones were not cytotoxic to the vaccine cells, but they inhibited proliferation of the vaccine cells. From the reactivity pattern of a panel of anti-clonotypic T cells towards a second panel of well characterized antigen specific T cells, it was concluded that the anti-clonotypic T cells may recognize at least two regions of the TCR: se-

TABLE I. T Cell Receptor Repertoire and Epitope Specificity of the MBP Reactive T Cell Clones Isolated Before and 2 Years After Vaccination[a]

	Clones isolated before vaccination							Clones isolated after vaccination[a]						
			TCR β junctional sequence Epitope						TCR β junctional sequence					
	Clone	Vβ	V	n-D-n	J	C	Epitope reactivity	Clone	Vβ	V	n-D-n	J	C	Epitope reactivity
Patient1	*3*	2.3	YFCA	ARGGPASE	ELFFGEGSRLTVL	EDLKN	84-102	2B3	18.x	YLCASS	SIWTGD	GYTFGSGTRTVV	EDLNK	110-129
		2.3	YFCA	ARGGPASE	ELFFGEGSRLTVL	EDLKN	84-102	1E11	18.x	YLCASS	SIWTGD	GYTFGSGTRTVV	EDLNK	110-129
								2D7	18.x	YLCASS	SIWTGD	GYTFGSGTRTVV	EDLNK	110-129
								1C9	18.x	YLCASS	SIWTGD	GYTFGSGTRTVV	EDLNK	110-129
								2C6	18.x	YLCASS	SIWTGD	GYTFGSGTRTVV	EDLNK	110-129
								2D3	3.1	YLCASS	QLQGA	YEQYFGPGTRLTVT	EDLKN	143-168
								2G9	3.1	YLCASS	QLQGA	YEQYFGPGTRLTVT	EDLKN	143-168
Patient 2	*D4*	5	YLCA	QDRVPK	NIQYFGAGTRLSVL	EDLKN	84-102	2F10	6.6	YLCASS	SGGTV	YGYTFGSGTRLTVV	EDLK	84-102
	D7	5	YLCA	QDRVPK	NIQYFGAGTRLSVL	EDLKN	84-102	2C5[b]	13.1	YFCAS	LGQGW	NTEAFFGQGTRLTVV	EDLKN	124-142
	B10	5	YLCA	QDRVPK	NIQYFGAGTRLSVL	EDLKN	84-102	2C10	13.1	YFCAS	RPGQL	NYGYTFGSGTRGTVV	EDLKN	61-82
	G9[b]	13.1	YFCAS	LGQGW	NTEAFFGQGTRLTVV	EDLKN	124-143	2C3	13.1	YFCAS	RPGQL	NYGYTFGSGTRGTVV	EDLKN	61-82
								1B7	13.1	YFCAS	RPGQL	NYGYTFGSGTRGTVV	EDLKN	61-82

[a]The clones were isolated 2 years after vaccination.
[b]Identical clones. The underlined are the clones used for immunization.
*In 3/8 vaccinated patients MBP reactive T cells reappeared in the circulation 2 years after vaccination. The TCR junctional sequences of the β-chains of the MBP reactive T cell clones isolated before and after vaccination are listed for two patients. The reappearing clones expressed TCR sequences different than those of the clones that were used for vaccination. In patient 2, clone 2C5 expressed the same TCR as clone G9, however the latter clone was not used for vaccination.

quences in the highly variable CDR3 region and a sequence in the less variable CDR2 region (Zhang et al., 1995b). In our ongoing experiments we are fine-mapping the TCR epitopes that are recognized by the anti-clonotypic cells in a given patient. Thus, our study suggests that these anti-clonotypic T cell lines recognize a TCR hypervariable region characteristic for the immunizing clones and an additional less variable V region sequence. These observations are of considerable importance to understand the in vivo clonotypic regulatory network, and in designing future T cell vaccination trials.

In conclusion, the study has confirmed in a clinical setting that T cell vaccination can be applied to boost clonotypic regulatory mechanisms in depleting pathologically relevant autoreactive T cells. We have recently demonstrated that significant anti-clonotypic T cell responses to the vaccine cells are still present 1 to 3 years after vaccination (Zhang et al., 1995b). Although MBP reactive T cells remain undetectable in the majority of treated patients 2–3 years after vaccination, in three out of eight patients MBP reactive T cells reappeared in the circulation. Remarkably, the reappearance of these cells coincided with clinical relapses in two of these patients (Zhang et al., 1995b; Medaer et al., 1995). The reappearing clones were found to originate from different clonal origins than those of the T cells that were used in the vaccine (Table I). Some patients were revaccinated with these clones to further deplete the MBP reactive T cells. Interestingly, the anti-clonotypic T cell responses induced by the vaccination were restricted to the immunizing clones and did not affect the reappearing MBP reactive T cell clones, nor T cell clones with different antigen reactivity, indicating the specificity of this approach. Some mechanisms of T cell vaccination, such as anti-ergotypic T cell responses, may however also induce less specific T cell immunity to the vaccine clones as discussed in a later section.

Clinical Data

Although the pilot study was not designed to draw conclusions as to the treatment efficacy, preliminary information on the therapeutic effects was obtained from the clinical data of the first eight patients, five patients with relapsing-remitting disease and three progressive patients. These patients were followed for at least 2 years after treatment. Before the study, these patients were paired to control MS patients. The control patients were matched for age, disease duration, disease subtype (relapsing-remitting or progressive disease), expanded disability status score (EDSS), and relapse rate. Clinical EDSS scores, frequency of relapses, and brain lesions as scored by magnetic resonance imaging (MRI) were evaluated in treated and control patients 2 years prior and two years after the treatment. In the 2 years before and after vaccination, the total number of exacerbations decreased from 16 to 3 in five vaccinated patients with relapsing-remitting disease, and from 12 to 10 in the matched control patients (Medaer et al., 1995). MRI showed a mean increase of 8.0% in brain lesion scores in the vaccinated patients compared to a 39.5% increase in the

controls. The brain lesion scores were determined based on the size and number of lesions as determined on T2 weighted images. Interestingly, the lesions and/or relapses worsened in three patients after vaccination in association with the reappearance of circulating MBP reactive T cells, as discussed above (Medaer et al., 1995). In three vaccinated patients with chronic progressive MS, no obvious effects on the clinical course were seen. Thus, our data suggest a moderate clinical improvement in some patients with relapsing-remitting MS who received T cell vaccination, as evidenced by a reduced rate of exacerbation and stabilization of disease scores and brain lesions. The clinical data therefore encourage larger double-blind trials to further prove the potential therapeutic effects of T cell vaccination.

THE MECHANISM OF T CELL VACCINATION

Since T cell vaccination has now entered clinical trials in human autoimmune diseases, it will be crucial to understand the immunological and physiological mechanisms of T cell vaccination. From the animal studies it was learned that the TCR is a major target of the anti-T cell response. T cells reactive against irrelevant control antigens did not induce resistance. From the lymph nodes of vaccinated animals both $CD4^+$ and $CD8^+$ T cell clones were obtained which influenced the in vitro proliferation of the vaccinated clone. The $CD4^+$ cells enhanced, while the $CD8^+$ clones suppressed, the proliferation of the clone used for vaccination (Lider et al., 1986). Since these anti-vaccine clones could not affect proliferation of irrelevant clones, the TCR is probably responsible for the specificity of this effect. In another study, a $CD8^+$ T cell clone was generated from vaccinated animals, which induced specific cytotoxicity towards the vaccine cells (Sun et al., 1988). In EAE, some experiments showed that autoreactive T cells were not eliminated but rather suppressed after vaccination (Naperstek et al., 1982). A recent study of T cell vaccination with T cells reactive to a heat shock protein (HSP) peptide in NOD mice illustrated an alternative effector mechanism of anti-idiotypic T cells in the vaccinated animals (Cohen et al., 1995). These anti-idiotypic T cells recognized an hypervariable V-D-J sequence of the TCR β chain of the autoreactive T cells. Interestingly, the vaccination induced a shift from a pro-inflammatory Th1 to a beneficial Th2 phenotype of the autoreactive T cells. How anti-idiotypic T cells can induce a shift in the cytokine profile of the autoreactive T cells is still an open question.

In addition to the highly specific anti-idiotypic responses, T cell vaccination in animal models also induces T cell responses towards other determinants of activated T cells. The cells were named anti-ergotypic cells and were stimulated by both syngeneic and allogeneic activated T cells. Upon transfer, these cells were able to suppress EAE (Lohse et al., 1989). The protective effects of this nonspecific response were however less pronounced as compared to those induced by the anti-idiotypic or anti-clonotypic cells. The target determinants of the anti-ergotypic T cells has not been defined yet.

In our study of T cell vaccination in MS we identified two subsets of anti-clonotypic T cells: $CD8^+$ and $CD4^+$ cells (Zhang et al., 1993). We have clearly shown that $CD8^+$ anti- clonotypic T cells are MHC I restricted and highly cytotoxic towards the vaccine cells. Because of their cytotoxic potential and the associated depletion of MBP reactive T cells after vaccination, it was assumed that elimination of autoreactive T cells was mainly induced by the lytic effects of the $CD8^+$ anti-clonotypic T cells. Alternatively, MBP reactive T cells could have been anergyzed by these cells and become undetectable in our frequency studies. In addition to the $CD8^+$ cells, we also identified a few $CD4^+$ anti-clonotypic T cells. The functional mechanism of these $CD4^+$ cells is not clear; they have only low cytotoxic potential. In analogy to similar T cell populations in patients treated with TCR peptides, these cells are suspected to induce T cell suppression or T cell anergy. To understand this mechanism, we are currently evaluating the cytokine profile of these clones.

It is not clear how the TCR of autoreactive cells is recognized by anti-clonotypic T cells, but the current hypotheses are depicted in Figure 2. TCR's are most likely presented as processed antigenic peptides by the T cells in association with either MHC class I or class II elements. Since MHC class II elements are induced on activated T cells, this could explain the need for the use of activated cells as vaccines. Furthermore, the TCR could also be released in a soluble form and taken up by other antigen presenting cells and presented by class II elements. Since $CD8^+$ anti-clonotypic T cells were predominantly observed in our study, the class I presentation pathway seems to be the most important in our vaccination protocol.

We have observed low, though significant T cell responses to activated T cells in almost all patients, and some 'anti-ergotypic' lines were isolated. It is not clear how this response contributes to the protective effects of the vaccination. Since these cells will recognize activated T cells, this response may be relevant in suppressing autoreactive T cells with specificity for other myelin antigens. Remarkably, in some patients we observed a significant γδ T cell response towards the vaccine cells (Stinissen et al., unpublished observation). In our current studies we are evaluating whether this γδ T cell response is an anti-ergotypic-like response and whether they play

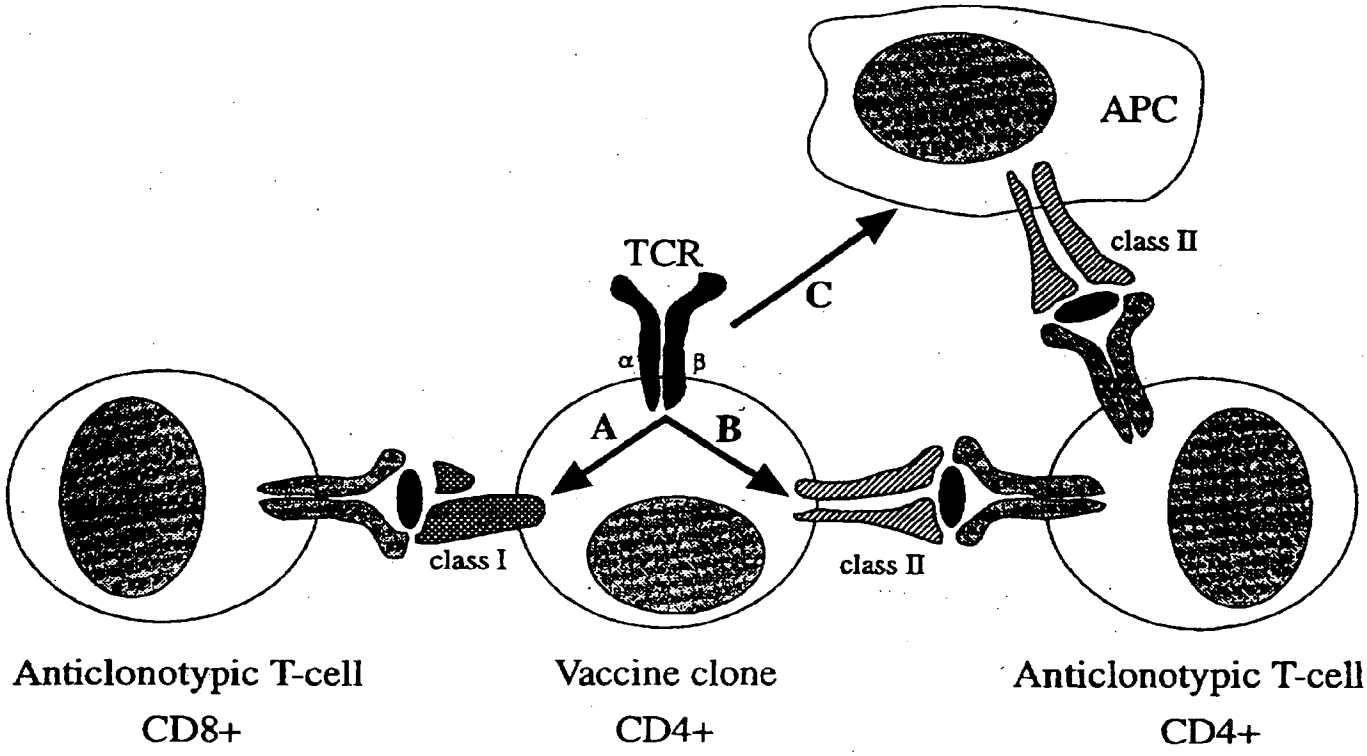

Fig. 2. Presentation of TCR peptides to anti-clonotypic T cells. TCR peptides are processed by the T cells and presented either by class I molecules to $CD8^+$ anti-clonotypic T cells (A), or by class II molecules to $CD4^+$ anti-clonotypic T cells (B). Alternatively, the TCR is released from the T cells, taken up by antigen presenting cells and presented in association with class II molecules to $CD4^+$ anti-clonotypic T cells (C).

an important role in the T cell vaccination mechanism. Finally, we believe that antibody responses play only a marginal role in the anti-vaccine responses. Indeed, even though a large number of serum samples were tested at different time-points a low titer of anti-T cell vaccine antibodies was observed in one patient only.

In conclusion, $CD8^+$ and $CD4^+$ anti-clonotypic T cells are probably the most important mediators of the functional mechanisms in vaccinated MS patients. They are directed to either hypervariable CDR3 or less variable CDR2 TCR regions, and induce specific cytotoxic ($CD8^+$) or T cell specific suppresser effects ($CD4^+$) in vaccinated subjects. Furthermore, other less specific responses to the vaccine clones may be regulated by anti-ergotypic T cells or other T cell subsets.

CONCLUSION AND SOME REMARKS FOR FUTURE STUDIES

Although several important issues were addressed in the present pilot trial, many questions remain to be answered. Some of these issues are related to the experimental protocol of the treatment, e.g. what is the optimal dose of the vaccine, what is the optimal method of attenuation, and which is the best route of administration (Cohen et al., 1995). Other questions are related to the fundamental design of the T cell vaccination, but may also be particularly relevant to other T cell directed therapies. For instance, which clones will be incorporated in the vaccine, or in other words, which clones will be the primary target of the T cell directed therapy? Since only activated clones can cross the blood brain barrier and become involved in the pathogenic cascade, our major focus will be targeted at these populations. Activated clones could be identified by selecting T cell lines after IL-2 stimulation (Zhang et al., 1994). An alternative approach is to identify the clonally expanded populations, since they are the result of in vivo activation (Vandevyver et al., 1995). There is some consensus that the 84-102 epitope is an important encephalitogenic region in $HLA\text{-}DR2^+$ patients. In future studies with large number of patients it will be crucial to rapidly identify myelin reactive clones that are relevant to the disease process. The rapid and detailed analysis of the patient's anti-MBP T cell repertoire will become increasingly important to allow a selection of relevant clones for vacci-

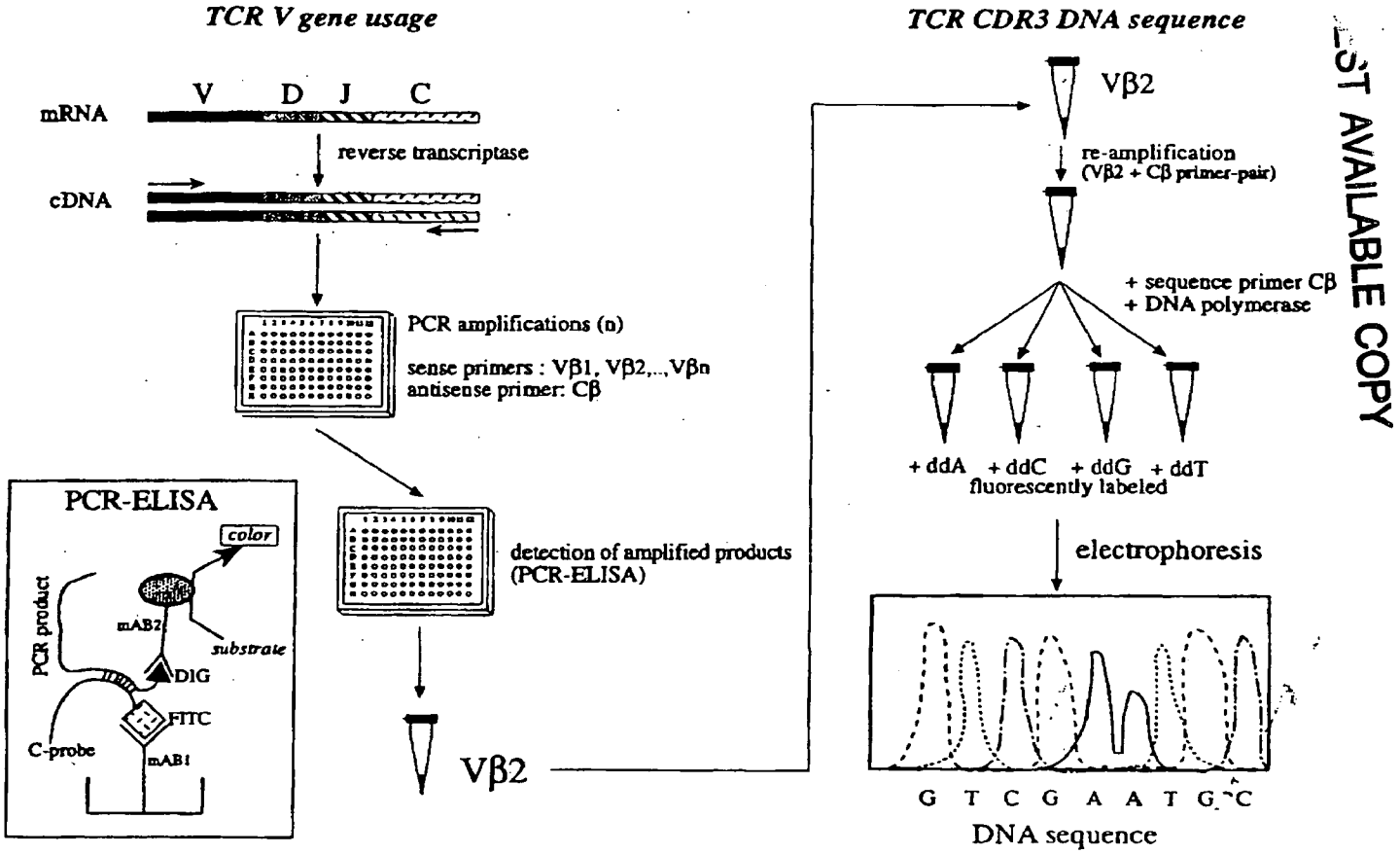

Fig. 3. Schematic overview of the procedure to determine the TCR Vβ gene usage and CDR3 sequences of the MBP reactive T cell clones. In future T cell vaccination or TCR peptide vaccination trials it will be crucial to obtain rapid information on the autoreactive T cell repertoire. For this purpose, we have optimized a fast method to determine the TCR characteristics of T cell clones. First, mRNA isolated from the T cells (0.2–1 million cells) is transcribed into cDNA using a commercial single-tube system. The cDNA is used as template in a panel of PCR amplifications with specific primers for each Vβ (or Vα) gene family. The sense primers bind to the various V gene elements, while the DIG labeled antisense primer is based on the Cβ (or Cα) gene sequence. The PCR reactions are performed in microtiter plates and the amplified products are identified by a fast ELISA based technique. In this method, the specific PCR products are denatured and hybridized to FITC-labeled C-β (or Cα) probes. These DNA hybrids, carrying both DIG and FITC labels, are first bound to an anti-FITC antibody (mAB1) coated on a microtiter plate, and in a secondary reaction an anti-DIG antibody (mAB2) binds to the immobilized PCR products. The enzyme activity of the peroxidase enzyme linked to the Fc part of the anti-DIG allows colorimetric detection of the specific PCR products. The identified V gene element is further expanded in a second round of PCR, and sequenced using fluorescently labeled dideoxynucleotides. The sequence reaction products are analyzed in an automated sequence-reader. This method allows determination of TCR CDR3 DNA sequences in less than 48 hr.

nation. Independent T cell clones representing the pre-vaccination repertoire can be obtained from almost all subjects (>95%) within 2 months. To allow rapid identification of clonally expanded populations optimized methods have to be used to determine the Vα-Vβ gene usage and sequence the TCR junctional regions of the MBP reactive T cell clones. As illustrated in Figure 3, we combine PCR in microtiter plates, detection of PCR products by ELISA, and automated sequencing, allowing determination of TCR DNA sequences within 48 hr.

Another important issue is related to the MBP reactive T cells that are isolated from vaccinated patients, 1 to 3 years after vaccination. Interestingly, these patients (three of eight) underwent a clinical deterioration and a brain lesion worsening as evidenced by MRI at the time when circulating MBP reactive T cells reappeared. The results suggest that in these cases the three events, exacerbation, worsening of MRI lesions and reappearance of MBP reactive T cells, may be related to a common pathological reactions that induced the clinical relapses. These observations further support the role of MBP reactive T cells in the pathogenesis of MS. Thus,

after depletion of the dominant clones, other clones that were cryptic before vaccination were activated and induced a clinical exacerbation in these patients. Alternatively, these clones might have been missed by the initial repertoire determination. Although these clones can be depleted in a second round of vaccination, it will be of extreme importance to carefully select the vaccine clones as discussed above. The data from this study may also be relevant to other TCR based specific therapies, where T cells are targeted based on their TCR determinants.

The antigen specificity of the vaccine clones is an important point of discussion. The activation status, epitope restriction, clonal expansion and persistence of MBP reactive T cells suggest an important role for MBP as a primary target antigen in MS. However, the reactivity towards other myelin antigens, including PLP and MOG may also be important in the pathogenesis of MS (Markovic-Plese et al., 1995; Kerlero de Rosbo et al., 1993). At least two mechanisms may account for the heterogeneous myelin antigen recognition in MS. First, different myelin antigens may trigger the initial autoimmune response in distinct patients, depending on the nature of the cross-reactive viral epitope or superantigen and MHC background. Second, the observed antigen recognition profile may represent the intermolecular dispersion of an immune response that arose initially against a single component of myelin. Hence, although the initial response is directed at MBP, the breakdown of the blood brain barrier and continuous demyelination may release other myelin antigens leading to the activation of T cells reactive to other myelin components. Epitope spreading has been observed in the EAE model, and this phenomenon may limit the success of antigen specific immune therapies in MS (Lehman et al., 1992, 1993). However, several authors have reported the persistence of MBP reactive T cells specific for the immunodominant 84-102 epitope in some HLA-DR2$^+$ MS patients for several years, indicating that in these patients the anti-myelin response remains dominated by a single or a few T cell clones (Meinl et al., 1993, Salvetti et al., 1993; Wucherpfennig et al., 1994; Vandevyver et al., 1995; Hohlfeld et al., 1995). Therefore, these clones could be the target of successful immune therapies. Alternatively, patients sensitized to various myelin antigens, for instance PLP, MOG, or MBP could be preselected, and appropriate antigen-specific T cell vaccination could be performed in these patients. Of course, this would significantly complicate this approach.

Finally, the current concepts on the pathogenesis of MS suggest that antigen specific T cell responses are more relevant in the early phases of the disease, therefore T cell vaccination and other antigen specific therapies are considered to be more effective in early MS patients.

In conclusion, our study is the first to apply antigen reactive T cell clones as vaccines in a human T cell vaccination trial in MS. This method appeared to be effective in depletion of circulating MBP reactive T cells in all patients. The preliminary clinical data showed encouraging effects in the vaccinated patients with relapsing remitting disease. More definite results on the treatment efficacy will be offered by double blinded studies with a larger number of patients. One obstacle associated with the current vaccination protocol remains the necessity to prepare a vaccine for each individual patient. At this time, it is difficult to predict whether T cell vaccination will remain a personalized treatment, or may be generalized using (a) peptide(s) in a category of patients with defined MHC alleles, whose targeted autoreactive T cells share a common TCR structural feature. In a more generalized form of T cell vaccination synthetic peptides or related T cell membrane fractions containing a desired target sequence may be used. The key question is whether a target sequence seen by anti-clonotypic T cells in one individual may trigger the same clonotypic regulation in another. Further studies need to resolve whether relevant MBP-specific T cell clones isolated from the peripheral blood as well as cerebrospinal fluid of MS patients with a given MHC haplotype may display a limited motif within the V-(D)-J junctional region of their α or β chains.

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